

The Incorporation of Chromium in Rice Straw Fermented with *Ganoderma lucidum*

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ABSTRACT

The research was designed to evaluate chromium incorporation by *Ganoderma lucidum* in rice straw substrate supplemented with chromium chloride. Treatments were combination of Cr level (0, 500, 1000, 1500, 2000, 25000, and 3000 ppm) and fermentation time (0, 2, 3, and 4 wk). The treatments were arranged in a factorial 7 x 4 and allocated in completely randomized design with three replications. *G. lucidum* was grown in potato dextrose agar (PDA) medium for 10 days and was inoculated to the substrate which have been sterilized and mixed with $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$. The moisture of substrate was maintained at 70%. Fiber and protein components of growth media of *G. lucidum* was determined and analyzed for their Cr content. The finding result showed that addition of Cr up to 3000 ppm into the substrate stimulated the *G. lucidum* growth. Chromium was incorporated into the fiber and protein components of the growth substrate of *G. lucidum* during fermentation. Incorporation of Cr into the protein of substrate containing 3000 ppm Cr was highest when fermented for 4 wk. Protein component of substrate contained 9.29% Cr while in NDF and ADF was 27.20% and 10.55% Cr, respectively. It is concluded that Cr was incorporated into the *G. lucidum* cells during fermentation.

Key words: chromium, *Ganoderma lucidum*, incorporation, rice straw

ABSTRAK

Penelitian dirancang untuk mengevaluasi inkorporasi kromium oleh fungi *Ganoderma lucidum* pada substrat jerami padi yang ditambah dengan kromium klorida. Perlakuan pada penelitian ini adalah kombinasi kadar chromium (0, 500, 1000, 1500, 2000, 2500, dan 3000 ppm) dengan lama fermentasi (0, 2, 3, dan 4 minggu). Perlakuan dirancang dengan menggunakan pola faktorial (7 x 4) yang dialokasikan dalam rancangan acak lengkap dengan tiga ulangan. Starter *G. lucidum* ditumbuhkan pada media PDA (potato dextrose agar) selama 10 hari dan dinokulasikan pada substrat yang telah ditambah dengan $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ dan diautoclave. Kadar air substrat ditetapkan 70%. Komponen serat dan protein dari media pertumbuhan *G. lucidum* ditentukan dan dianalisa kandungan Cr-nya. Hasil penelitian menunjukkan bahwa penambahan Cr sampai 3000 ppm pada substrat merangsang pertumbuhan *G. lucidum*. Kromium terinkorporasi ke dalam komponen serat dan protein pada substrat pertumbuhan *G. lucidum*. Inkorporasi Cr ke dalam protein substrat yang mengandung 3000 ppm Cr dan difermentasi selama 4 minggu lebih tinggi dibandingkan kadar lainnya. Komponen protein mengandung 9,29% Cr sedangkan di dalam NDF and ADF masing-masing adalah 27,20% dan 10,55% Cr. Dapat disimpulkan bahwa Cr terinkorporasi ke dalam sel miselium *G. lucidum* selama fermentasi.

Kata kunci: chromium, *Ganoderma lucidum*, inkorporasi, jerami padi

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INTRODUCTION

Chromium (Cr) is one of the micro mineral and its essentiality has been proved in many researches. Chromium is important for animals (NRC, 1997) because of their role in the metabolism of carbohydrates, fats, proteins and nucleic acids (Pechova & Pavlata, 2007). Chromium from inorganic sources is poorly absorbed. The conversion of inorganic Cr to an organic complex improves its bioavailability (NRC 1997; Pechova & Pavlata, 2007). Supplementation of mixed Cr in fermented feed and *G. lucidum* improved immune response of lactating cows (Agustin *et al.*, 2011). Therefore, efforts should be made to convert inorganic Cr to organic form. Effective Cr level for *G. lucidum* growth in solid state fermentation of oil palm by-product was 3000 ppm with incorporation value of 68.23% (Agustin *et al.*, 2010). Metal ions including Cr can accumulate intracellularly in fungal cells (Sen & Dastidar, 2010). The ability of microorganism cell to bind metal ions is associated with its cell wall (Shugaba *et al.*, 2012).

Ganoderma lucidum is a species of Basidiomycetes that can utilize fiber as a substrate for its growth (Chang & Miles, 2004). *G. lucidum* which belongs to the white rot fungi can produce extracellular enzymes laccase, which has a high ligninolytic activity (Baldrian, 2003; Chang & Miles, 2004; Mtui & Nakamura, 2007; Ke *et al.*, 2011). The fungi are able to tolerate the toxic effects of hexavalent Cr (Prasenjit & Sumathi, 2005; Shugaba *et al.*, 2012) and generally show greater resistance to metal ion concentration than many bacterial species (Oliver *et al.*, 2005; Ezzouhri *et al.*, 2009; Sen & Dastidar, 2010).

G. lucidum is able to use sawdust as a substrate for its growth (Chang & Miles, 2004) and it can also use oil palm by product for its growth (Agustin *et al.*, 2010). A source of fiber such as rice straw is available and its availability is abundant. Rice straw may meet the criteria as a source of fiber for *G. lucidum* growth. Misra *et al.*, (2007) reported that mustard straw fermented by *G. lucidum* at a temperature of 35 °C for 21 d can increase *in vitro* digestibility and delignification. *G. lucidum* can bind and can accumulate metal ions intracellularly. However, information about the use of rice straw as a growth medium of *G. lucidum* for the purpose of producing organic Cr have not available yet. The experiment was designed to determine the incorporation of Cr by *G. lucidum* in rice straw as a main component of substrate. The result obtained is an effort to synthesize organic Cr to be used as a mineral supplement for livestock. In this case the whole fermented substrate is used as the product.

MATERIALS AND METHODS

Preparation Starter of *Ganoderma lucidum*

Inoculum stock of *G. lucidum* was obtained from the collection of Indonesian Biotechnology Research Institute for Estate Crops (IBRI), Bogor. Starter was obtained by rejuvenating inoculums stock at IBRI Microbial Laboratory and Laboratory of Microbiology, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University. The

rejuvenation of inoculums was carried out by culturing isolates of stock culture *G. lucidum* into petridish containing PDA medium (potato-dextrose-agar) (Wagner *et al.*, 2003), and was incubated for 10 d or until the mycelium of *G. lucidum* met petridish. The production of the inoculums was a crucial step affecting significantly the performance of the subsequent fermentation.

Substrate Preparation and Inoculation

The main material used in this research was derived from rice straw obtained from rice fields. Rice straw was chopped to the size of 2-3 cm and dried in the sun to the moisture content of about 10%. Rice straw was mixed with a solution containing $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ with varying Cr concentration: 0, 500, 1000, 1500, 2000, 2500, and 3000 mg Cr per kg of dry matter and conditioned at 70% moisture content. The mixed substrate was inserted into the 300 ml bottle, covered with aluminum foil, and sterilized for 30 min at pressure of 1.2 and temperature of 121 °C. After cooling, sterilized bottle were inoculated with starter aseptically with a plug diameter of 0.5 cm in the laminar flow cabinet. The inoculated substrate was incubated at 27-28 °C for 0, 2, 3, or 4 wk. Fermentation products were harvested according to the time of incubation.

Experimental Design

The experiment was carried out in a 7 x 4 factorial design with 3 replications for each treatment. There were seven levels of Cr supplemented into the rice straw, *i.e.* 0, 500, 1000, 1500, 2000, 2500, and 3000 ppm, at the time factor. There were four fermentation times as the second factor, *i.e.* 0, 2, 3, and 4 wk.

Sample and Data Analysis

Percentage of dry matter (DM) and organic matter (OM) loss were calculated. Total Cr in the fermented biomass was measured by using Atomic Absorption Spectrophotometer (AAS) Shimadzu AA680 models (Carry & Allaway, 1971). The amount of Cr bound fiber and protein components of biomass was measured separately. Measurement of total Cr in solid substrate was conducted by using a sample of 5 g. Samples were crushed in a mortar porcelain and put into a test tube, add 5 mL of 10% TCA and was homogenized with a vortex, and then allowed to stand for one hour. The solution was then centrifuged at 3000 rpm for 10 min. Chromium concentration in supernatant was measured. The content of the neutral detergent fiber fraction of fiber (NDF) and acid detergent fiber (ADF) were determined by analysis of Van Soest (Van Soest & Wine 1967). Levels of Cr contained in the NDF and ADF are determined from the sample after the determination of NDF and ADF. Biomass protein content was analyzed (Bradford 1976) and its Cr levels were measured.

The data were analyzed statistically according to two-way Analysis of Variance (ANOVA) and the difference between treatment means was determined using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

G. lucidum grew rapidly on PDA on the fifth until 10 d of incubation. The mycelium reached maximum growth on stable condition when the level of water was 70%. The water content of the rice straw substrate in this study was higher than recommended by Chang & Miles (2004) which was 60%-65%. This is because the substrate for cultivation of *G. lucidum* was different. Chang & Miles (2004) used saw dust as a substrate, while in this study we used rice straw as a main component of substrate. The growth of *G. lucidum* varied widely, depending on the kind of substrate (Erkel, 2009). Fermentation with *G. lucidum* was carried out at temperatures ranged from 26-28 °C and pH of substrate 5.5. The optimum fermentation condition appeared to depend on temperature and pH, and this substrate conditions was suitable for the growth of *G. lucidum* according to the recommendation of Chang & Miles (2004). The growth of fungi *G. lucidum* on rice straw substrate containing up to 3000 ppm Cr began to appear after the age of 1 wk. After 2 wk of fermentation, its white mycelium had covered half of the substrate surface of the media in the bottle. Mycelium growth in 3000 ppm Cr levels up to 4 wk of incubation was excellent but has not formed fruiting bodies.

Dry Matter and Organic Matter Content of Fermented Rice Straw

Dry matter content of fermented rice straw was influenced by the fermentation time and Cr levels ($P<0.01$) but there was no interaction between the two factors (Table 1). Dry matter content declined sharply in the second week of fermentation, from 29.24% to 26.5%, suggesting that the nutrients contained in the substrate has been used by fungi.

Dry matter content of substrate continued to decrease up to 4 wk of incubation. It was suggested that the nutrients in rice straw was used by *G. lucidum* to increase the number of cells as indicated by the growth of mycelium. There was a linear relationship between the dry matter content of rice straw media with fermentation

time according to the equation, $Y = -1.11 X + 29.70$ ($R^2 = 0.8053$, $P < 0.01$ where Y = change in the levels of dry matter, %; X = length of fermentation, days). This means that as the fermentation time increased by one unit there was a reduction of dry matter by 1.11%. Ligninolytic enzyme produced by *G. lucidum* degraded lignin from the rice straw, and this was associated with fermentation time and the reduce of dry matter. Product of degraded lignin was used by *G. lucidum* to its mycelium growth.

Chromium supplementation appeared to decrease the rate of decline in dry matter content of rice straw substrate. Slowing decline in dry matter content of rice straw occurred at 1500 ppm Cr levels up to 3000 ppm. It is expected that high levels of Cr inhibits the growth of *G. lucidum* so that decomposition of organic material into H_2O and CO_2 slowed and ultimately decreased the proportion of dry matter of fermented rice straw. However, mycelium growth was not impaired as it was observed that mycelium growth covered the media even at Cr levels of 3000 ppm.

The rate of change in the content of organic matter of fermented rice straw was similar to that of dry matter, which was somewhat influenced by fermentation time and Cr levels ($P<0.01$) (Figure 1). A decline in organic matter content was observed with increasing duration of fermentation from 0 to 4 wk. The decline of organic matter content was from 23.95% to 19.55% in fermentation time of 4 wk. Oxidases such as laccase from *G. lucidum* are enzymes which were involved in oxidative conversions of organic compounds and materials (Mtui, 2012).

This result showed that the proportion of inorganic materials with organic media remains the same for all the different levels of Cr. It is thought that the formation of organic matter occurred in the form of mycelium and was comparable to the decreasing levels of organic matter or dry matter content.

The Loss of Dry Matter and Organic Matter in Fermented Rice Straw

The loss of dry matter and organic matter in fermented rice straw was significantly increased ($P<0.01$) with increasing length of fermentation time. There was

Table 1. The percentage of dry matter content fermented with *Ganoderma lucidum* on rice straw with Cr levels 0-3000 ppm and fermentation time 0, 2, 3, 4 wk

Cr level (ppm)	Fermentation time (Wk)				Average
	0	2	3	4	
0	29.27±0.31	26.66±0.49	25.27±0.23	24.87±1.35	26.52±1.91 ^a
500	28.75±0.63	26.72±0.67	25.84±0.27	24.57±0.85	26.46±1.68 ^a
1,000	28.80±0.86	25.29±0.47	25.85±0.28	25.25±0.24	26.30±1.59 ^a
1,500	29.86±0.63	26.72±0.73	26.81±2.01	25.29±2.08	27.17±2.17 ^b
2,000	29.02±0.40	26.54±0.63	26.35±0.80	26.04±0.74	26.99±1.36 ^b
2,500	29.09±0.65	26.69±0.27	26.44±0.58	26.01±0.79	27.06±1.35 ^b
3,000	29.90±0.50	26.89±0.21	27.85±0.98	27.10±0.01	27.93±1.33 ^c
Average	29.24±0.67 ^c	26.50±0.68 ^b	26.34±1.11 ^b	25.59±1.21 ^a	

Note: Means in the same row or column with different superscript differ significantly ($P<0.01$).

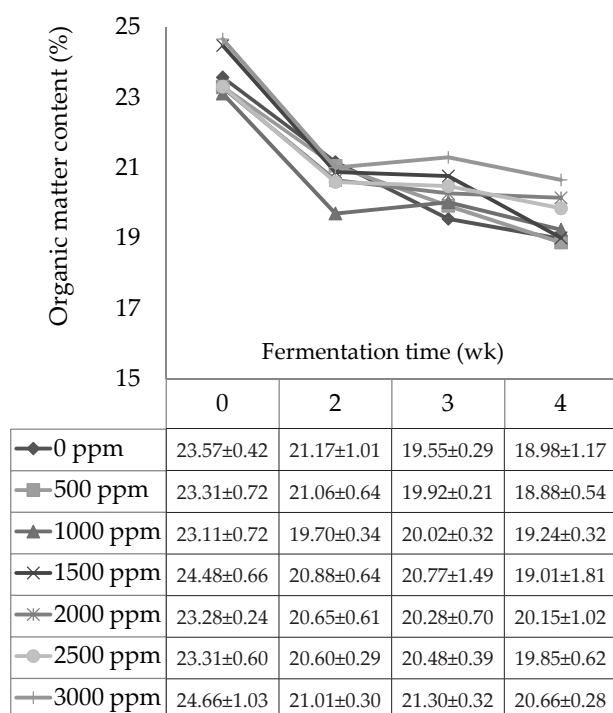


Figure 1. Curves percentage of organic matter content fermented with *Ganoderma lucidum* on rice straw substrate with Cr levels 0 (◆-), 500 (■-), 1000 (▲-), 1500 (x-), 2000 (*-), 2500 (-●-), and 3000 (-|-) ppm and fermentation time 0, 2, 3, and 4 wk.

no interaction between the fermentation periods with Cr levels (Table 2). Dry matter loss in rice straw increased with increasing duration of fermentation, followed by growth of the mycelium which filled the surface of the substrate and covered the growing medium. Dry matter loss begins in 2 wk (12.49%) and continued to increase up to 4 wk (17.19%). This condition suggests that with increasing dry matter loss, more nutrients were utilized by *G. lucidum* for the metabolism and growth of its mycelium. There was a linear relationship between the dry matter loss to the fermentation time of fermented rice

straw by following equation: $Y = 5.356X - 2.35$ ($R^2 = 0.83$, $P < 0.01$ where Y = dry matter loss, %, X = length of fermentation, week), but rate of dry matter loss decreases with increasing Cr at a level of more than 2000 ppm Cr. This suggests that the levels of more than 2000 ppm Cr decreased the growth rate of *G. lucidum*. In our research, the dry matter loss in fermented rice straw have the different profile with the dry matter loss in oil palm by-product fermented with *G. lucidum*. In contrast, in case of oil palm by-product, the increase of Cr level in substrate up to 3000 ppm Cr did not affect the growth rate of *G. lucidum* (Agustin *et al.*, 2010). The loss of dry matter on cultivation of oyster mushroom in wheat straw substrate was 9.4% (Pant *et al.*, 2006), while in this study, the loss of dry matter was 17.19%. This difference in result could be due to *G. lucidum* being a white rot fungus which is capable of producing ligninolytic enzyme (laccase, manganese peroxidase, lignin peroxidase) (Ke *et al.*, 2011) and able to degrade fiber components of rice straw.

The pattern of organic matter loss was the same with the dry matter loss in fermented rice straw in relation to fermentation time and levels of Cr in media. Means of organic matter loss in fermented rice straw was influenced by the fermentation time ($P < 0.01$) and Cr levels ($P < 0.05$). There was no interaction between Cr levels with long fermentation (Figure 2). Organic matter loss begins at week 2 (14.97%) and continued to increase until at week 4 fermentation period (21.88%). Increasing the rate of organic matter loss in rice straw showed an increase in the amount of nutrients utilized by *G. lucidum* for its metabolic activity and its cell growth.

At the early stage of fermentation, *G. lucidum* utilized the digestible nutrients contained in rice straw, after which the fungus utilized structural carbohydrates, especially complex compounds that are bound together with lignin. *G. lucidum* produces laccase enzyme which is capable of hydrolyzing lignin binding with other compounds (Baldrian, 2003; Ke *et al.*, 2011). Increased organic matter loss decreased with increasing fermentation time. These results indicated that *G. lucidum* was able to hydrolyze and utilize structural carbohydrates and lignin.

Table 2. The loss of dry matter of *Ganoderma lucidum* fermentation product (%) on rice straw substrate with Cr levels 0-3000 ppm and fermentation time 0, 2, 3, 4 wk

Cr level (ppm)	Fermentation time (Wk)				Average
	0	2	3	4	
0	0	11.96±2.45	17.31±0.98	20.12±4.62	12.35±8.36 ^b
500	0	10.05±4.15	14.59±2.64	19.26±4.69	10.97±7.98 ^b
1,000	0	14.95±3.81	13.85±1.58	16.52±2.24	11.33±7.19 ^b
1,500	0	13.31±1.19	15.19±6.76	20.77±5.30	12.31±8.78 ^b
2,000	0	11.31±2.26	14.93±4.02	15.17±1.28	10.35±6.76 ^a
2,500	0	11.21±2.28	12.67±3.15	14.63±1.62	9.63±6.21 ^a
3,000	0	14.64±8.10	11.00±2.24	13.87±0.79	9.88±7.10 ^a
Average	0 ^a	12.49±3.82 ^b	14.48±3.51 ^b	17.19±4.43 ^c	

Note: Means in the same row or column with different superscript differ significantly ($P < 0.01$).

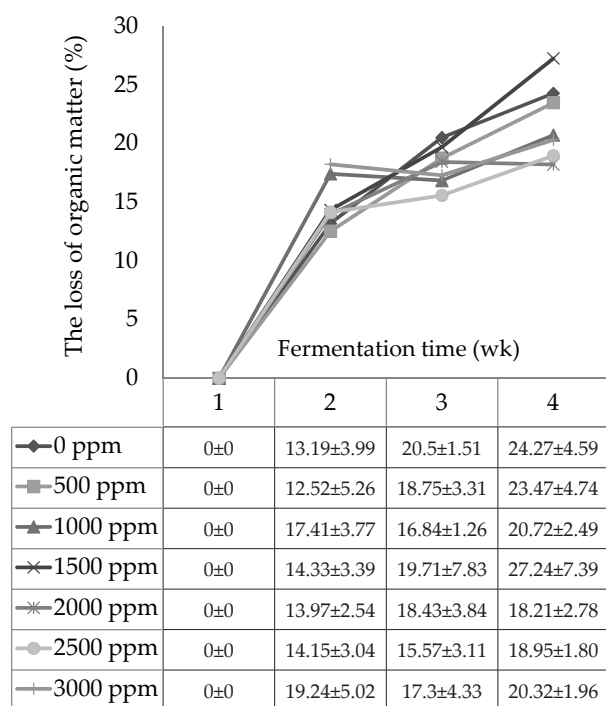


Figure 2. The loss of organic matter of *Ganoderma lucidum* fermentation product (%) on rice straw substrate with Cr levels 0 (—♦—), 500 (—■—), 1000 (—▲—), 1500 (—x—), 2000 (—*—), 2500 (—●—), and 3000 (—|—) ppm and fermentation time 0, 2, 3, and 4 wk.

Cr Incorporation in Fermented Products

The element Cr bound in media was probability bound with media components and was converted into mycelial cell component of *G. lucidum*. Chromium incorporation into the cell of *G. lucidum* (Figure 3) is thought to occur because it grew well in the rice straw substrate. The binding and incorporation of Cr was significantly affected ($P < 0.01$) by the Cr levels of substrate. There was no interaction between Cr levels with fermentation time. *G. lucidum* using Cr together with the use of organic matter of rice straw fiber components. This was indicated by the amount of Cr bound and incorporated in fermentation product. Incorporated Cr in fermentation product of *G. lucidum* was increase along with the higher Cr levels in substrate. The binding and incorporation of Cr in Cr levels of 1000 ppm was only 120 ppm Cr. The amount of incorporated Cr increase continuously until its value 1410.82 ppm Cr at the Cr levels of 3000 ppm based on dry matter. There was a linear relationship between the binding and incorporation of Cr with a Cr content of the substrate following the equation: $Y = 296.72X - 471.86$ ($R^2 = 0.98$, $P < 0.01$ where Y = Cr bound, %; X = Cr content of the media, 500 ppm). This result showed that *G. lucidum* was more resistance to supplementation of Cr in high concentration because mycelia growth gave the organism a larger surface area, which provides greater protection to sensitive organelles of the fungi. In addition, fungi are eukaryotic cells, which

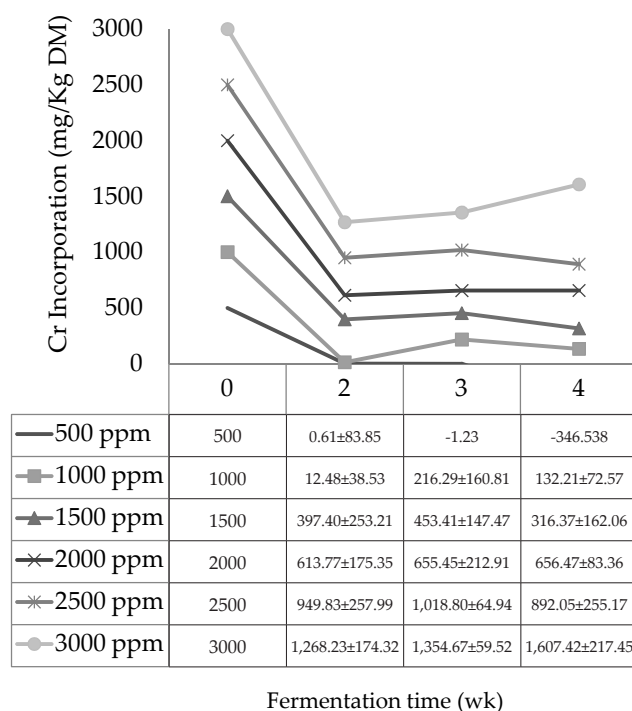


Figure 3. Incorporation of Cr on *Ganoderma lucidum* cell in rice straw substrate with Cr levels 500 (—), 1000 (—*—), 1500 (—♦—), 2000 (—●—), 2500 (—x—), and 3000 (—■—) ppm and fermentation time 2, 3 and 4 wk.

contain more genes that can inhibit toxic metals (Sen & Dastidar, 2010; Shugaba *et al.*, 2012).

The highest Cr level supplemented into rice straw substrate was 3000 ppm. The highest Cr incorporation value was also found on substrate with Cr level of 3000 ppm (Figure 3). The element Cr was needed in the nutrient metabolism of carbohydrates and protein of *G. lucidum* cell for 4 weeks of fermentation. This indicates that *G. lucidum* was able to adapt to the medium with high levels of Cr. *G. lucidum* was also able to use the media components that are difficult to degrade.

G. lucidum was able to incorporate Cr into its cell proteins with the value of 9.29% (Table 3) when cultured in substrates containing 3000 ppm Cr. Some Cr was deposited in the neutral detergent fibre (NDF) (27.20%) and acid detergent fibre (ADF) (10.55%). These results showed that there has been a conversion of inorganic to organic forms of Cr by *G. lucidum*. Cr founded in the protein of *G. lucidum* cell is an organic compound. The highest efficiency of Cr incorporation was 53.58% showed in treatment with 3000 ppm Cr and fermentation time of 4 wk (Figure 3). It has been suggested that chromium ions can be incorporated into *G. lucidum* cells by two step, the first step involves the surface binding of Cr (III) ions to the cell wall and extracellular material, which is not depend on metabolism process. The second step is depending on the metabolic activity because uptake Cr (III) into the cell across the cell membrane and at that time is occurred intracellular accumulation (Sen & Dastidar, 2010).

Table 3. The number of Cr in fiber component and protein of *Ganoderma lucidum* fermentation product on rice straw containing 3,000 ppm Cr in the form $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$

Parameter	Dry matter (g)	Cr level (mg/kg DM)	Total Cr (mg)
Total products	235.6	3030	714
NDF	173	1112	194
ADF	157	480	75
NDF + ADF	380	1592	269
Protein	0.895	74112	66

CONCLUSION

The loss of dry matter and organic matter in fermented rice straw was related to the growth of mycelium of *G. lucidum*. The binding of Cr occurs by fiber matrix of substrate. Inorganic Cr conversion into organic forms by fungi *G. lucidum* on rice straw substrate was evidenced by the presence of Cr in protein of fermented product. Protein component of fermented product contained 9.29% Cr while in NDF and ADF was 27.20% and 10.55% Cr, respectively at level of 3000 ppm Cr. Chromium was incorporated into the *G. lucidum* cells during fermentation and *G. lucidum* is tolerant of high Cr levels up to 3000 ppm.

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